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Frank Witte

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EXAMINER

NGUYEN, QUANG

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

DETAILED ACTION

Applicant's amendment filed on 1/4/2010 was entered.

Amended claims 1-12, 14, 18 and new claim 21 are pending in the present application, and they are examined on the merits herein.

Priority

The present application is a 371 of PCT/EP04/11287, filed on 10/08/2004, which claims benefit of the provisional application 60/509,942, filed on 10/10/2003, and the foreign application EPO 03022780.5, filed on 10/10/2003.

Upon review of the specifications of the provisional application 60/509,942 and the foreign application EPO 03022780.5, and comparison with the specification of the present application, it is determined that the instant claims are only entitled **to the effective filing date of 10/08/2004** because both the provisional application 60/077,262 and the foreign application EPO 03022780.5 do not have a written support for the concept of cultivating chondrocytes at unphysiologically high extracellular concentration of magnesium (Mg), wherein said unphysiologically high extracellular concentrations of Mg range up to 20 mM, and characterized in that at least once a first unphysiologically high extracellular Mg concentration is increased to a second unphysiologically high extracellular Mg concentration during cell cultivation which promotes proliferating chondrocytes to form chondrons.

Claim Objections

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Claims 3 and 10 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. This is because the recited range of extracellular concentrations of Mg from about 12 mMol to about 65 mMol and of 11 to 25 mMol, in claims 3 and 10, respectively, is outside of the limitation "said unphysiologically high extracellular concentrations of Mg range up to 20 mM" recited in independent claim 1 from which both claims 3 and 10 are dependent on.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

An embodiment of claims 3 and 10 (unphysiologically high extracellular concentrations of Mg outside the Mg concentration range recited in independent claim 1) are still rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for the generation of chondrons comprising the step of **cultivation of chondrocytes** at unphysiologically high extracellular concentrations of magnesium (Mg), characterized in that at least once an unphysiologically high extracellular Mg concentration is increased during the cultivation, and **wherein said high extracellular concentrations of Mg range up to 20 mM;**

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does not reasonably provide enablement for **a method for the generation of chondrons using any other cells and/or at any other unphysiologically high extracellular concentrations of Mg as broadly claimed**. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims for the same reasons already set forth in the Office action mailed on 4/01/2009 (pages 2-7).

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 1/4/2010 (page 4) have been fully considered but they are moot because apart from an embodiment of claims 3 and 10 which recites unphysiologically high extracellular concentrations of Mg outside the Mg concentration range recited in independent claim 1 (e.g., {Mg}> 20 mM), the above rejection was withdrawn for all other claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of

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the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-2, 5 and 7-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caruso (US 4,978,661) in view of Egerbacher et al. (Vet Pathol 38:143-148, 2001; Cited previously) for the same reasons already set forth in the Office action mailed on 4/01/2009 (pages 8-10). ***The same rejection is restated below.***

Within the scope of enablement, Caruso discloses a method of treating rheumatoid arthritis comprising administering to a patient, including intra-articularly injecting, a therapeutically effective amount of 6-halo-4-quinolone derivatives (see at least the abstract and issued claims). Caruso also teaches that the “**multilocal**” **intra-articular treatment** with 6-halo-4-quinolone derivatives induce at least the clinical remission of the early rheumatoid illness (col. 3, lines 60-68; col. 4, lines 1-6). Caruso further discloses that 6-halo-4-quinolone derivatives are already known in the art and they are described as antimicrobial agents useful in the treatment of urinary and respiratory infections (col. 2, lines 22-31).

Caruso did not teach a method of culturing chondrocytes in the presence of an unphysiologically high extracellular concentration of magnesium, and wherein at least

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once the unphysiologically high extracellular Mg concentration is increased during the culture.

However, at the effective filing date of the present application Egerbacher et al already taught that magnesium supplementation at 1X concentration (0.0612 mg/ml MgCl + 0.0488 mg/ml MgSO₄ = 1mM MgCl + 0.4 mM MgSO₄) or at 3X concentration (about 4.2 mM Mg) has a significantly positive or protective effect on quinoline-treated horse and dog chondrocytes in 5-day cultures, with more positive effects observed for a triple dose (see at least the abstract; Figures 1-5; page 144, col. 1, second paragraph; and section titled "Magnesium supplementation"). Egerbacher et al further disclosed that the addition of Mg²⁺ slightly increased cell proliferation (53% for Mg1 and 55% for Mg3) with respect to cells cultivated in Mg²⁺-free medium (47%; see page 146, col. 1, second paragraph; Figure 6).

It would have been obvious for an ordinary skilled artisan to modify the teachings of Caruso by also intraarticularly injecting magnesium and maintaining magnesium (including repeated injections) at a concentration within a range of 1.4 mM to 4.2 mM for the arthritic joint of a patient treated with 6-halo-4-quinolone derivatives in light of the teachings of Egerbacher et al.

An ordinary skilled artisan would have been motivated to carry out the above modification because Egerbacher et al already showed at least protective effects of Mg supplementation at a concentration within a range of 1.4 mM and 4.2 mM for horse and dog chondrocytes against quinolones in tissue cultures, and that Mg²⁺ supplementation also slightly increased cell proliferation.

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An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Caruso and Egerbacher et al.; coupled with a high level of skill for an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 1/4/2010 (pages 5-6) have been fully considered but they are respectfully not found persuasive for the following reasons.

Applicants argue basically that the Egerbacher reference does not overcome the defects of the primary Caruso reference in any way because Egerbacher teaches the use of a single level of Mg, and that is taught as used only with quinolone-treated horse and dog chondrocytes. Egerbacher does not teach that cells which are cultivated at unphysiologically high extracellular concentrations of magnesium, should be subjected at least once to a change from an original or first unphysiologically high concentration to an even higher or second unphysiologically high concentration of Mg concentration (e.g., from 5 mM to 10 mM Mg). Egerbacher lacks any teaching or suggestion of an additional increasing step to convert the cells from the proliferation phase to the differentiation phase as demonstrated by the examples of the present patent application and as required in claim 1.

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First, it appears that Applicants considered the teachings of Caruso and Egerbacher references in total isolation one from the other. Of course, please note that since the above rejection was made under 35 U.S.C. 103(a) neither the Caruso nor the Egerbacher reference has to teach every limitation of the instant claims.

Second, as written there is no recitation whatsoever about different chondrocyte cultivation phases such as the proliferation phase and the differentiation phase as argued by Applicants. The claims simply require the cultivation of chondrocytes in both in vitro and/or in vivo at unphysiologically high extracellular concentrations of Mg, in which a first unphysiological high extracellular Mg concentration is increased to a second unphysiologically high extracellular Mg concentration during cell cultivation, and wherein said unphysiologically high extracellular concentrations of Mg range up to 20 mM.

Third, as already stated in the above rejection it would have been obvious for an ordinary skilled artisan to modify the teachings of Caruso by also intraarticularly injecting magnesium and maintaining magnesium (including **repeated injections**) at a concentration within a range of 1.4 mM to 4.2 mM for the arthritic joint of a patient treated with 6-halo-4-quinolone derivatives because Egerbacher et al already showed at least protective effects of Mg supplementation at a concentration within a range of 1.4 mM and 4.2 mM for horse and dog chondrocytes against quinolones in tissue cultures, and that Mg²⁺ supplementation also slightly increased cell proliferation. By repeating intraarticular injecting magnesium to maintain a magnesium concentration of at least 1.4 mM in order to attain the protective effects taught by Egerbacher et, this step satisfies

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the limitation "a first unphysiologically high extracellular Mg concentration is increased to a second unphysiologically high extracellular Mg concentration". Additionally, please note that Caruso teaches explicitly a treatment protocol that involves repeated injections (see at least col. 3, lines 60-68). Furthermore, it should also be noted that "a first unphysiologically high extracellular Mg concentration" could be an unphysiologically high extracellular Mg concentration at 1h, 3hrs, 5hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs...after an initial unphysiologically high extracellular concentration of Mg was injected.

Accordingly, claims 1-2, 5 and 7-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caruso in view of Egerbacher et al. for the same reasons already set forth in the Office action mailed on 4/01/2009 (pages 8-10).

Claims 1-2, 4-9, 11-12, 14, 18 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Masuda et al. (US 2001/0012965) in view of Egerbacher et al. (Vet Pathol 38:143-148, 2001; Cited previously), Halvorsen et al. (US 6,841,150), and Lindenberg et al (US 2005/0239040). ***This is a modified rejection necessitated by Applicant's amendment to accommodate amended claims 11-12 and new claim 21.***

Within the scope of enablement, Masuda et al already disclose at least a method for producing a transplantable cartilage matrix, comprising culturing isolated chondrogenic cells, including human adult articular chondrocytes and bovine chondrocytes, in alginate culture containing a stimulatory agent, such as fetal bovine

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serum and/or exogenously added specific growth factors such as osteogenic protein-1, TGF-beta, insulin like growth factor, for an amount of time effective for allowing formation of a chondrogenic cell-associated matrix (see at least Summary of the Invention; and particularly paragraphs 33-40 and example 1).

Masuda et al did not teach a method of culturing isolated chondrogenic cells in the presence of unphysiologically high extracellular concentrations of magnesium, wherein at least once a first unphysiologically high extracellular Mg concentration is increased to a second unphysiologically high extracellular Mg concentration during the cell culture; and/or the cultured chondrocytes are differentiated from adult stem cells; and/or the cultivation is effected under an oxygen partial pressure of 8%.

However, at the effective filing date of the present application Egerbacher et al already taught that **magnesium supplementation at 1X concentration (0.0612 mg/ml MgCl + 0.0488 mg/ml MgSO4 = 1mM MgCl + 0.4 mM MgSO4) or at 3X concentration (about 4.2 mM Mg) has a significantly positive or protective effect on quinoline-treated horse and dog chondrocytes in 5-day cultures, with more positive effects observed for a triple dose** (see at least the abstract; Figures 1-5; page 144, col. 1, second paragraph; and section titled "Magnesium supplementation"). Egerbacher et al further disclosed that **the addition of Mg²⁺ slightly increased cell proliferation (53% for Mg1 and 55% for Mg3) with respect to cells cultivated in Mg²⁺-free medium** (47%; see page 146, col. 1, second paragraph; Figure 6).

Additionally, Halvorsen also disclosed at least a method for directing adipose-derived stromal cells cultivated *in vitro*, including in a calcium alginate or another

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biocompatible lattice or matrix capable of supporting chondrogenesis in a three dimensional configuration, to differentiate into functional chondrocytes in conditions such as at temperatures between 31 °C to 37 °C in a humidified incubator, with a carbon dioxide content to be maintained between 2% to 10% and the oxygen content between 1% and 22% (see at least Summary of Invention; particularly col. 5, line 54 continues to line 7 of col. 6; col. 6, lines 61-65).

Moreover, at the effective filing date of the present application Lindenberg also taught an in vitro culture method for obtaining a mature oocyte in which the oxygen tension, a cell culture parameter, is regulated via a temporal rise or a temporal decrease in oxygen tension in one or more times (see at least the abstract and paragraphs 89-100,154-159).

It would have been obvious for an ordinary skilled artisan to modify the teachings of Masuda et al by also culturing and maintaining isolated chondrogenic cells (including a temporal rise in the extracellular Mg concentration in one or more times) in the presence of a high extracellular concentration of Mg ranging between 1.4 mM and 4 mM and/or using chondrocytes differentiated from adult stem cells as well as cultivating chondrocytes under an oxygen partial pressure of 8%, in light of the teachings of Egerbacher et al, Halvorsen et al and Lindenberg as discussed above.

An ordinary skilled artisan would have been motivated to carry out the above modification because Egerbacher et al already showed at least protective effects of Mg supplementation at a concentration within a range of 1.4 mM and 4.2 mM for horse and dog chondrocytes against quinolones in tissue cultures, and that Mg²⁺

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supplementation also slightly increased cell proliferation. Additionally, Halvorsen et al already taught successfully a cultivation of adipose-derived stromal cells in a calcium alginate culture to differentiate into functional chondrocytes as well as culturing differentiated chondrocytes with an oxygen content between 1% and 22%. Furthermore, Lindenberg already taught at least a cell culture parameter such as the oxygen parameter can be regulated via a temporal rise or a temporal decrease in one or more times in a cell culture.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Masuda et al, Egerbacher et al, Halvorsen et al and Lindenberg; coupled with a high level of skill for an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 1/4/2010 (pages 6-7) have been fully considered but they are respectfully not found persuasive for the following reasons.

Once again, Applicants argue basically that the Egerbacher reference does not overcome the defects of the primary Masuda reference in any way because Egerbacher teaches the use of a single level of Mg, and that is taught as used only with quinoloine-treated horse and dog chondrocytes. Egerbacher does not teach that cells which are

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cultivated at unphysiologically high extracellular concentrations of magnesium, should be subjected at least once to a change from an original or first unphysiologically high concentration to an even higher or second unphysiologically high concentration of Mg concentration (e.g., from 5 mM to 10 mM Mg). Egerbacher lacks any teaching or suggestion of an additional increasing step to convert the cells from the proliferation phase to the differentiation phase as demonstrated by the examples of the present patent application and as required in claim 1. The Halvorsen reference provides no teaching whatsoever of the significance of using higher than physiologically normal extracellular Mg concentrations, and in particular there is no teaching of shifting such a high extracellular Mg concentration to a still higher concentration during incubation in order to promote chondrocyte differentiation. Lindenberg reference does not deal with chondrocytes or with Mg concentrations in any way.

First, it appears that Applicants considered each of the cited references in total isolation one from the other. Of course, please note that since the above rejection was made under 35 U.S.C. 103(a) none of the cited references has to teach every limitation of the instant claims.

Second, once again there is no recitation whatsoever about different chondrocyte cultivation phases such as the proliferation phase and the differentiation phase as argued by Applicants. The claims simply require the cultivation of chondrocytes in both in vitro and/or in vivo at unphysiologically high extracellular concentrations of Mg, in which a first unphysiological high extracellular Mg concentration is increased to a second unphysiologically high extracellular Mg concentration during cell cultivation, and

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wherein said unphysiologically high extracellular concentrations of Mg range up to 20 mM.

Third, as already stated in the above 103 rejection it would have been obvious for an ordinary skilled artisan to modify the teachings of Masuda et al by also culturing and maintaining isolated chondrogenic cells (including a temporal rise in the extracellular Mg concentration in one or more times) in the presence of a high extracellular concentration of Mg ranging between 1.4 mM and 4 mM, because Egerbacher et al already showed at least protective effects of Mg supplementation at a concentration within a range of 1.4 mM and 4.2 mM for horse and dog chondrocytes against quinolones in tissue cultures, and that **Mg²⁺ supplementation also slightly increased cell proliferation.** Additionally, Halvorsen et al already taught successfully a cultivation of adipose-derived stromal cells in a calcium alginate culture to differentiate into functional chondrocytes as well as culturing differentiated chondrocytes with an oxygen content between 1% and 22%. Furthermore, **Lindenberg already taught at least a cell culture parameter such as the oxygen parameter can be regulated via a temporal rise or a temporal decrease in one or more times in a cell culture.** The citation of the Halvorsen reference is to demonstrate that at the effective filing date of the present application, chondrocytes can be differentiated in a culture in vitro from an adipose-derived stromal cell (at least an adult stem cell; see limitation of claim 7) and that differentiated chondrocytes were cultured with an oxygen content between 1% and 22% (limitation of claim 12). The citation of the Lindenberg reference is to demonstrate that at the effective filing date of the present application at least a cell culture parameter such as

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the oxygen parameter can be regulated via a temporal rise in one or more times in a cell culture; and accordingly in the combined teachings of Masuda et al, Egerbacher et al, Halvorsen et al and Lindenberg as set forth above, the desired unphysiologically high Mg concentrations can be similarly regulated.

Accordingly, claims 1-2, 4-9, 11-12, 14, 18 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Masuda et al. in view of Egerbacher et al., Halvorsen et al. and Lindenberg et al for the reasons set forth above.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Jeschke et al (US 2002/0052044) disclosed culturing human chondrocytes with human serum, as well as cultivating alginate-encapsulated chondrocytes under an oxygen partial pressure of 20% or less (e.g., 0.5 to 20%) (paragraphs 7-13).

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

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A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Voitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

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/QUANG NGUYEN/

Primary Examiner, Art Unit 1633

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